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Response of sooty blotch and flyspeck complex to optimum temperature and post-harvest removal on apples by brushing combined with dip treatments

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Signatures have been redacted for privacy

This thesis is dedicated to my parents Alfonso and Gladis.

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ABSTRACT

Sooty blotch and flyspeck (SBFS) is a serious late-season disease complex that colonizes the cuticle of apples (*Malus* x *domestica* Borkh). SBFS causes considerable economic loss to growers by reducing the market value of fruit. In addition, management of SBFS causes indirect economic losses due to the cost of frequent applications of protectant fungicides.

Little is known about responses of recently discovered members of the SBFS complex to environmental factors. The optimal temperature for mycelial growth of two isolates of each of five common Midwestern SBFS species (*Dissoconium* sp. DS1, *Colletogloeum* sp. FG2, *Peltaster* sp. P2, *Peltaster* sp. CS1, and *Pseudocercosporella* sp. RH1) and one previously described North Carolina species (*Peltaster fructicola* P1) was determined *in vitro* growth chambers. The isolates were evaluated at 10, 15, 20, 25, 30 and 35°C in growth chambers for 7 weeks in darkness. Mycelial growth was estimated by measuring the diameter of each colony every 7 to 11 days. Generally, optimum growth occurred at 20 to 25°C for all six species, with slower growth at 10 and 15°C and little to no growth at 30 or 35°C. Determining the temperature responses for SBFS complex increased our understanding of the fungi ecology and can contribute to development of more effective disease management strategies.

One feasible alternative to reduce SBFS to market-acceptable levels is post-harvest washing and brushing. The effectiveness of five post-harvest dip treatments (NaOCl at 200 and 500 μg/ml, ClO_2 at 1 and 5 μg/ml, and fruit soap) were evaluated for removal of SBFS on apples. After a 7-minute dip treatment, apples were brushed for 15, 30, 60, or 90 s on a grading line. Percent disease was determined before and after treatment of 'Honey Gold'

apples from Iowa and Wisconsin in 2002 and 'Golden Delicious' apples from Kentucky and North Carolina in 2003. Removal of SBFS in all treatments was variable, but generally exceeded the no-dip control. Increasing the brushing time significantly increased removal of SBFS signs. Post-harvest SBFS removal treatments therefore may provide growers with alternatives for improving appearance of SBFS – blemished apples to meet fresh-market standards.

CHAPTER 1. GENERAL INTRODUCTION

Thesis organization

This thesis contains an abstract and four chapters. The first chapter is a general introduction to the taxonomy, biology, ecology, and management of SBFS and concludes with the rationale and objectives for the research presented herein. The second chapter describes experiments to determine temperature optima for mycelial growth of SBFS on apples. The third chapter presents experiments using post-harvest brushing and dip treatments to remove SBFS signs from apples. The fourth chapter summarizes the research and provides overall conclusions to the thesis. References cited within each section are presented at the end of each chapter.

Literature review

Overview of the sooty blotch and flyspeck (SBFS) fungal complex

Sooty blotch and flyspeck is caused by a diverse group of saprophytic fungi that colonize the cuticle of apples (*Malus* x *domestica* Borkh.) and pears (*Pyrus communis* L.), resulting in cosmetic damage and reducing the quality and market value of the fruit. All cultivar of apples are considered susceptible to SBFS (24, 31, 69).

Sooty blotch and flyspeck signs

The SBFS fungi do not penetrate the apple cuticle or epidermis (11, 16, 46, 57). The growth of SBFS on apple surface appears to be induced by exudation of nutrients or accumulation of external substrates that retain fungal inoculum (11). The severity of SBFS signs varies among apple cultivars due to considerable differences in morphology of the

fungi, as well as differences in texture, thickness, morphology, quantity, and chemical composition of epicuticular wax. (11, 32).

Sooty blotch fungi form dark olive green to brown mycelial mats that vary in appearance and size (0.5 to 1.0 cm in diameter), ranging from small circular colonies to large colonies with diffuse margins. Sooty blotch fungi include dark, sclerotium-like bodies within the mycelial mat (16, 37). Flyspeck fungi appear as groups of tiny (1 to 3 cm in diameter), round to irregular-shaped, shiny black specks on the epicuticular wax (14, 16, 20, 33, 46, 51). SBFS signs are visible to the naked eye; differentiation of mycelium types, however, often requires microscopic evaluation (31).

SBS fungi differ in prevalence, incidence and severity among orchards (33, 37, 71). Mycelial types are more readily distinguished on apples with green or yellow skin than on apples with red skin (31, 36). On russeted cuticles, mycelial development is either inhibited or develops irregularly, resulting in coalescence of colonies (11, 75).

Sooty blotch and flyspeck worldwide distribution

In the United States, SBFS is most severe in the East, Midwest and Southeast during the late summer (74). SBFS has been also reported in Jamaica, Canada, Europe (Italy, France, England, Germany, Poland, and Serbia), Africa (Congo and South Africa), Australia, New Zealand, and Asia (China and Japan) (16, 31, 44, 45, 67).

Hosts of sooty blotch and flyspeck

Although economic losses caused by SBFS have been concentrated on pomaceous fruits, a wide range of wild plants surrounding orchards apparently can serve as alternative hosts of SBFS fungi (31, 34, 74). For example, sooty blotch was found in 25 species of

nonpomaceous trees and shrubs in Missouri (20), and Colby reported fungi on twigs and stems of 23 different trees in Indiana that exhibit morphology similar to that of sooty blotch fungi described on pome fruit (16).

The SBFS fungus *Geastrumia polystigmatis* has been reported to colonize common blackberry (*Rubus argutus* Link) in North America (34), the ornamental tree *Andira jamaicensis* in the Dominican Republic, and the tropical herb spiral ginger (*Costus afer*) in Tanzania (50). Similarly, *Leptodontidium elatius* has been reported in Nigeria, Great Britain, Canada, France and Germany on diverse species, including cottonwood (*Populus* spp.), spruces and pines (*Abies* spp.), paper birch (*Betula* spp.), peach (*Prunus* spp.), and hemlock (*Pseudotsuga* spp.) (19). *Peltaster fructicola* has been found on blackberry stems (36). *Zygophiala jamaicensis* has been reported in a wide range of hosts including leaf speckle of banana, greasy blotch of carnation, blackberry, grape, Japanese persimmon, Chinese quince, plum, pear, pawpaw and honeysuckle; the most common hosts were *Rubus* spp. (3, 21, 44, 46, 68).

Taxonomy of SBFS

The SBFS complex has received several common names over the years, including fruit spot, ink spot, flyspeck, sooty fungus, sooty mold, sooty spot, sooty blotch, and cloud. To date, however, the taxonomy of SBFS has been poorly characterized.

SBFS began to receive scientific attention in the early 1800s when growers noticed that the disease resulted in loss of quality and a reduction of customer acceptance in the market. Introduction of SBFS to the United States may have occurred when apples were imported from Europe in the 1600s (10).

Sooty blotch description. Sooty blotch was initially described on apples in 1832 and classified as a single fungus, *Dothidea pomigena* Schw. (56). The first description of sooty blotch colony morphology was provided by Sprague: "a dark, clouded stain, generally round, from a half to quarter of an inch in diameter" (64). Sprague changed the original name, *Dothidea pomigena,* to *Asteroma pomi* Schw. In 1883, the genus name for the causal agent of sooty blotch was again changed from *Asteroma pomi* to *Phyllachora pomigena* Schw., which became the accepted genus name for sooty blotch until the early 1900s (54). Colby (1920) revised the taxonomy of the sooty blotch and flyspeck complex. Colby associated *Phyllachora pomigena* with sooty blotch and renamed the species *Gloeodes pomigena* (Schw.) Colby.

Gloeodes pomigena (Schw.) Colby was considered the only causal agent of sooty blotch on apples until quite recently. In 1996, Johnson and Sutton (1997) suggested that sooty blotch was a complex caused by at least three fungal species. They classified the species according to colony types: *Leptodontidium elatius* (G. Mangenot) de Hoog from the fuliginous mycelial type, *Peltaster fructicola* Johnson, Sutton & Hodges from punctate, and *Geastrumia polystigmatis* Batista & M. L. Farr. from ramose. Sutton and co-workers were unable to find a fungus that matched the original description of *G. pomigena* (34, 35, 36, 74, 75).

Flyspeck description. Montagne and Fries named *Labrella pomi* Mont. Mss. (Fr. In litt.) as the causal agent for flyspeck on pear (45). However, Saccardo (53, 55) reassigned *Labrella pomi* to *Leptothyrium pomi* (Mont. & Fr.) Sacc. In 1896, Powell described the morphology of the causal agent of flyspeck, *Leptothyrium pomi* (Mont. & Fr.) Sacc., as "blackbirds" or "flies" (51). Four years later, Selby proposed that *Leptothyrium pomi* (Mont.

& Fr.) Sacc. was the sole causal agent of flyspeck (58). Toward the end of the 20th century, the accepted names for sooty blotch and flyspeck were *Gloeodes pomigena* and *Leptothyrium pomi*, respectively.

In 1930, Baines concluded that flyspeck produced thyriothecia, and renamed the causal fungus *Microthyriella rubi* Petrak (4). In 1959, Von Arx (73) reclassified the causal agent of flyspeck as *Schizothyrium pomi* (Mont. & Fr.) v. Arx. on maple. In 1945, Mason described *Zygophiala jamaicensis* on banana, but it was revealed to be the anamorph of *S. pomi* by Durbin in 1953 (21, 44).

Recently, Batzer and co-workers examined morphology as well as internal transcriber spacer (ITS) and large subunit (LSU) regions of ribosomal DNA (rDNA) for several hundred SBFS isolates from nine Midwest U.S. apple orchards (7). She described 30 putative species of SBFS on the basis of genetic and morphological distinctions and also noted the presence of *Peltaster fructicola* and *Zygophiala jamaicensis,* which had also been identified previously from North Carolina orchards. Analysis of LSU regions placed all SBFS species within the class Dothideomycetes; 27 species belonged to the order Dothideales, one to Pleosporales, and two were unclassified. The LSU sequences of 17 Dothideales species grouped with LSU sequences of known species of the genus *Mycosphaerella*. A similar phylogenetic analysis of SBFS apple isolates from orchards in Shaanxi Province, China, revealed 10 additional putative species and concluded that these species belonged to the order Dothideales (67).

Mycelial types. Colby (1920) was the first to describe the fern-like, honeycomb, and reticulate colony morphology types associated with SBFS. Further work by Groves in 1933 re-described the three colony types reported by Colby and renamed them, based on colony morphology on the apple cuticle and in culture. These types include: ramose (RS) (larger,

shinier, darker and more convex sclerotium-like bodies at the center of the colony than at the margin); punctate (P) (small, circular sclerotium-like bodies, comprised of several overlapping hyphal stands, that appear similar to the mycelial mats); ridged honeycomb (RH) (interspersed clumps and ridges of mycelia); and fuliginous (FG) (uniform mycelial mats; edges of the colony vary from abrupt to feathered) (7, 35, 36).

Batzer (7) recently described three morphological types of flyspeck-like fungi, having fruiting bodies without mycelial mats, on Midwestern apples: flyspeck (FS), having dark, shiny, round, and flattened sclerotium-like bodies; compact speck (CS), with shiny black, flattened sclerotium-like bodies, round to irregular, and densely arranged; and discrete speck (DS), having tiny, less dense spheres per unit surface area.

Environmental conditions that influence SBFS colonization of apples

Inoculation of apple fruit by SBFS fungi can occur at any time during fruit development, but mycelial growth occurs primarily late in the season. Generally, the incubation period during the spring or early summer was between 8 and 12 days under optimum conditions, but at least 3 weeks were required for development of signs in the field during late April (4, 69). However, mycelium can proliferate rapidly on apples when fungicide residues fall below effective levels after growers cease spraying for the season, or because environmental conditions become favorable for sporulation of SBFS (6, 36).

High rainfall, high relative humidity, and mild air temperatures are favorable for sporulation of SBFS fungi from early June through September (4, 6, 16, 31, 37, 69, 71). Disease severity is highest in wet years, especially during September and October (15, 41). In

the northern U. S., signs typically appear after the last fungicide spray in late August and peak at the beginning of September (4, 6, 31).

Ecology of SBFS

Studies have been conducted *in vitro* to determine the effect of relative humidity and temperature on SBFS fungi (4, 24, 31, 37, 41), as well as incidence and severity of SBFS on apples (15).

Temperature. It has been reported that growth of SBFS isolates occurred over a wide range of temperatures (5 to 27°C), optimum growth occurred between 15 and 24°C, and no growth at 0°C or 35°C (4, 5, 24, 31).

Growth and development of two common species of sooty blotch in North Carolina, *Peltaster fructicola* and *Leptodontidium elatius,* were studied *in vitro* to compare their response to temperature and relative humidity (37). Germination of conidia of *L. elatius* occurred between 12 and 32°C, whereas conidia of *P. fructicola* germinated only from 12 to 24°C. The optimum temperature for mycelial growth was 12 to 24°C for *P. fructicola* and 16 to 28°C for *L. elatius* (37).

Greatest conidia production of *Zygophiala jamaicensis* was observed from 16 to 20°C, whereas ascospores germinated at 16 to 28°C. Optimum mycelial growth occurred from 16 to 24°C (48). The temperature range at which maturation and development of *Zygophiala jamaicensis* spores occurred was 9 to 21°C (41). Similarly, Baines (1940) reported an optimum temperature range of 15 to 24°C and little growth was observed at 27°C.

Relative humidity. Relative humidity (RH) greater than 95% is required for growth of

mycelium, and germination of ascospores and conidia of *Zygophiala jamaicensis* (17, 48). Lerner (1999) observed spore development of *Zygophiala jamaicensis* at 99% relative humidity. Sharp and Yoder reported that flyspeck required periods of relative humidity above 95% for signs to appear, approximately 3 weeks after the last fungicide application (59). Development of sooty blotch signs was not affected by rainfall; in contrast, flyspeck severity was greater during wetter seasons (48). Germination *in vitro* of conidia of *P. fructicola* occurred at relative humidity of 93 to 95%, whereas conidia of *L. elatius* germinated at 97 to 99% RH (37).

Isolation and growth of SBFS

SBFS fungi are difficult to isolate because they grow slowly in culture and are frequently overgrown by contaminating microorganisms. SBFS fungi have been grown in oatmeal agar, corn-meal agar, potato dextrose agar, malt extract agar, apple juice agar, water agar, V-8 agar, and carnation leaf agar (4, 41, 72, 75).

Morphological characteristics, mycelial growth, and conidial production of SBFS isolates differ among media due to genetic and physiological differences among species (31, 72, 75). Spores of many SBFS fungi can be produced on either malt extract agar, potato dextrose agar (PDA), or V-8 agar (4, 41).

Most isolates of SBFS reach a colony diameter of only 10 to 15 mm on various agar media in 21 days (4, 5, 35, 36). Average growth of 30 isolates of *Zygophiala jamaicensis* from Massachusetts, New York, North Carolina, Kentucky, and West Virginia was measured *in vitro* on PDA and V-8 agar. The average diameter growth of cultures of all isolates was 27.4 mm on PDA and 30.0 mm on V-8 agar after 28 days at 21°C (41).

Economic impact of SBFS

SBFS can reduce the fresh market value of apples by more than 90% (74). For example, a bushel of certain premium varieties of apples may sell for as much as $32 on the fresh market, whereas a bushel of apples affected by SBFS, which is suitable only for making cider, may sell for as little as $2 (8).

In addition to the cosmetic damage caused by SBFS, the disease can produce indirect losses due to the cost of frequent applications of protectant fungicides (52). In order to suppress SBFS, fungicides are typically sprayed every 10 to 14 days from the first-cover stage and until shortly before harvest (9, 30, 27, 70). In warm, humid regions, however, even weekly applications of fungicides are frequently inadequate to control SBFS, resulting in a 5 to 10% annual reduction in marketable apples (43). In the Southeast U. S., losses of almost 100% were observed in orchards where protectant fungicides were not applied (71).

Management of SBFS

As a result of significant quality losses in pome fruits, several investigations have focused on improving SBFS management strategies (36). Quality loss of apples infested with SBFS increased significantly where the disease was not well managed (36). Disease incidence also increased in orchards with poor air drainage due to slow drying after rain and dew (16, 52). SBFS is often managed with protectant fungicides; however, apple growers have experienced control failures due to inadequate coverage of fungicide, deficient pruning, and disease-favorable environment (17, 37).

Pruning. The selective removal of branches and shoots facilitates drying of the canopy, thereby creating an unfavorable environment for SBFS (33). Pruning reduces

inoculum and improves fungicide coverage (17). Appropriate pruning is essential to reduce SBFS risk during wet seasons (16, 49). Reduction of approximately 50% of flyspeck was observed in summer-pruned apple trees where no fungicides were applied (17).

Fungicides. Historically, use of fungicides to control SBFS can be categorized into four sequential phases: (i) inorganic fungicide application, (ii) organic fungicide application, (iii) restriction of fungicide applications, and (iv) model-based fungicide applications (74).

Bordeaux mix, a mixture of copper sulfate and calcium carbonate, was the first fungicide used against SBFS, in 1894 (39). By spraying every 2 to 4 weeks during the season, the incidence of this disease could be reduced from 78% to 18% (40). By 1910, lime sulfur was being used commonly due to its lower phytotoxicity (31).

With the introduction of organic fungicides, such as the monoalkyldithiocarbamate fungicide ferbam and the phthalamide fungicide captan, in the early 1950s, SBFS signs seemed to reappear due to less persistence of these chemicals compared to inorganic fungicides, as well as inadequate practical information about how to use the new materials (25, 74). Hickey (1960) found that ferbam was more effective on sooty blotch and less effective on flyspeck than Bordeaux mix. Captan was found to fail in controlling sooty blotch due to its short residual activity (31). In order to increase the residual activity, captan was combined with lead arsenate, a common insecticide, which increased the residual activity by 25 days (31, 42). In light of these studies, it was recommended to spray every 10-14 days during the summer.

Ethylene bisdithiocarbamate (EBDC) fungicides, which have greater residual activity against SBFS fungi, were introduced to apple spray programs in the 1960s and widely used through the early 1990s (74). Zineb alone provided up to 60 days of residual activity,

whereas mancozeb provided 20-30 days more protection for sooty blotch and 30-50 days more protection for flyspeck than captan (13). Mancozeb or captan combined with benomyl decreased the development of SBFS symptoms (14). Resurgence of SBFS, especially in the Southeastern U.S., was seen twice in the 1970s due to the loss of lead arsenate, and in the early 1990s due to restrictions on use of EBDC fungicides (74). The first resurgence was controlled by increasing the gallonage per acre of fungicide applications, whereas the second resurgence was controlled by new organic fungicides such as the benzimidazoles benomyl and thiophanate-methyl, preferably in combination with ziram or captan (74).

For various chemicals with eradicant properties, spray programs were developed in conjunction with models to better time the application of the benzimidazole fungicides, benomyl plus metiram, and combinations of captan or mancozeb with benomyl (15, 27, 28, 29, 60, 61, 62, 63).

Currently, the fungicides used most commonly against SBFS include benzimidazoles (benomyl, thiophanate-methyl), captan, dithiocarbamates (ziram, thiram, ferbam), and strobilurins (kresoxim-methyl, trifloxystrobin) (74).

Warning system. For human health, fungicide resistance management, and environmental health, SBFS warning systems were developed in North Carolina and Kentucky. These programs extend the period between the 1st-cover and 2nd-cover fungicide sprays (13, 27, 28, 61). Validation trials conducted in the upper Midwest demonstrated that warning systems could save an average of 2.5 fungicides sprays per season compared to a conventional calendar-based spray schedule (1, 22, 23).

Timing of the first application is based on reaching a threshold number of hours of leaf wetness accumulated since the first-cover spray (10 days after petal fall). The threshold

varies according to the version of the warning system used. In the Northeast, for example, the spray threshold was 300 hours of leaf wetness, whereas in the Southeast fungicides were applied after 250 hours of leaf wetness (33). Brown and Sutton (1995) suggested an interval of 200 to 250 hours of leaf wetness to initiate fungicide application for the control of SBFS in North Carolina.

Post-harvest treatments. The SBFS complex does not influence growth and development of the fruit, but after a long period of storage, apples with SBFS develop a shriveled appearance. In early studies, evidence for growth and dissemination of SBFS among apples stored at low temperatures was not found (66). However, disease development is often related to the length of time between the last fungicide application and harvest.

Post-harvest removal of signs is another alternative to control SBFS. This tactic could allow apple growers to reduce fungicide applications and compensate for inadequate control of the disease in the orchard. Efficient use of sanitizers such as chlorine dioxide (ClO_2), sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), peroxyacetic acid, and fruit soap could increase fruit quality and fresh market grade of fruit and vegetables through removal of bacteria and fungi (8, 12, 30, 65, 74).

Sodium hypochlorite reduces SBFS severity by oxidation (8, 16, 74). Colby (1920) studied the effect of Javelle water (sodium hypochlorite) to remove SBFS. Apples were dipped from 3 to 6 minutes, then washed and rinsed with tap water.

Chlorine dioxide is a powerful sanitizer that has a wide antimicrobial spectrum (38) and is also an effective sporicide (26). Chlorine was introduced by Baker (1932) to control apple decay. Chlorine at 50 μg/ml and chlorine dioxide at 10 μg/ml significantly reduced

conidial germination of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* on d' Anjou pear (65).

Hendrix reduced the incidence of sooty blotch from 100 to 0% and flyspeck from 100 to 27% when apples were dipped in 500 ppm of chlorine combined with brushing for 5 to 7 minutes, then rinsed with tap water (30).

Effectiveness of post-harvest dip treatments [200, 400, 500, 600, or 800 ppm chlorine, sodium hypochlorite (Agclor 310 plus Decco 312 Buffer), a mixture of hydrogen peroxide and peroxyacetic acid (Tsunami 100) at 60ppm/80ppm, 120 ppm/160ppm, or 360ppm/480ppm, respectively, or fruit soap (Kleen 440)] and dip time (7 or 15 minutes) were evaluated for SBFS removal from Golden Delicious and Jonathan apples. A 7-minute dip in 800 ppm chlorine increased market value of Jonathan apples by 14% and Golden Delicious by 31%, and a 200 ppm chlorine dip resulted in 28 and 45% increase in market value after treatment for Jonathan and McIntosh, respectively (8).

The goals of the present study were to increase ecological understanding of this fungal complex while providing apple growers with alternative management strategies to improve the market value of their apples. The objectives of the study were to 1) determine the optimum temperature for mycelial growth of newly discovered fungi in the SBFS complex and 2) assess impact of post-harvest dip treatments and brushing time on removal of SBFS from apples.

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CHAPTER 2. TEMPERATURE OPTIMA FOR MYCELIAL GROWTH OF SOOTY BLOTCH AND FLYSPECK FUNGI ON APPLES

A manuscript prepared for submission to *Plant Disease*

Abstract

Over 30 fungal species cause sooty blotch and flyspeck (SBFS), an epiphytic complex resulting in cosmetic damage that reduces the value of apples. Little is known about responses of recently discovered members of the SBFS complex to environmental factors. The optimal temperature for mycelial growth of two isolates of each of five common Midwestern SBFS putative species (*Dissoconium* sp. DS1, *Colletogloeum* sp. FG2, *Peltaster* sp. P2, *Peltaster* sp. CS1, and *Pseudocercosporella* sp. RH1) and one previously described species from North Carolina (*Peltaster fructicola* P1) was determined in growth chambers. These isolates were evaluated at 10, 15, 20, 25, 30 and 35°C for 7 wk in darkness. Mycelial growth was estimated by measuring the diameter of each colony every 7 to 11 days. Optimum growth occurred at 20 to 25°C for all six species, with slower growth at 10 and 15°C and little to no growth at 30 or 35°C. Determining the temperature responses for SBFS complex increased our understanding of the fungi ecology and can contribute to development of more effective disease management strategies.

Introduction

The sooty blotch and flyspeck (SBFS) complex is a group of saprophytic fungi that colonize the cuticle of apples (*Malus* x *domestica* Borkh). Sooty blotch results in dark olive green to brown blemishes (9, 21), whereas flyspeck appears as groups of tiny, round to irregular-shaped, shiny black specks (6, 9, 11, 22, 26, 29).

SBFS-infested apples cause considerable direct economic loss to growers due to the low acceptance of blemished apples by consumers (9, 18, 22, 23, 29). In addition to the cosmetic effects caused by SBFS, the disease can produce indirect losses due to the cost of frequent applications of protectant fungicides (30). In order to suppress SBFS, fungicides in the Midwest U. S. are typically sprayed three to eight times, on a calendar-based schedule, from shortly after bloom stage until shortly before harvest (14).

Several studies have been conducted to determine the optimum temperature and relative humidity for germination of conidia and growth of mycelium of SBFS fungi (2, 15, 18, 21, 23). Growth of these isolates occurred over a wide range of temperatures (5 to 28°C), but optimum growth occurred between 15 and 24°C and no growth at 0°C or 35°C (2, 3,15, 18, 21, 27).

Until recently, only four SBFS species were recognized: *Leptodontidium elatius* (G. Mangenot) de Hoog (10), *Peltaster fructicola* (Johnson, Sutton & Hodges) (19, 20), *Geastrumia polystigmatis* (Batista & M. L. Farr.) (28), and *Zygophiala jamaicensis* Mason (24). Batzer and co-workers (5) examined the morphological and molecular characteristics of several hundred SBFS isolates from nine Midwest apple orchards and identified 30 new species of SBFS. However, nothing is known about temperature responses of these newly discovered isolates. This information could provide a better understanding of the ecology of the SBFS complex and potentially lead to more effective management practices. For example, knowledge of temperature optima for growth and sporulation of these fungi could ultimately be used in warning systems to help growers time fungicide sprays more efficiently. The objective of this study was to determine the influence of temperature on

mycelial growth of five newly discovered SBFS fungi and one previously named SBFS fungus.

Materials and methods

Sources of isolates. Two isolates each of *Dissoconium* sp. DS1, *Colletogloeum* sp. FG2, *Peltaster fructicola* P1, *Peltaster* sp. P2, *Peltaster* sp. CS1, and *Pseudocercosporella* sp. RH1 were selected. Mycelial type, isolate designation, and origin are summarized in Table 1. All except *Peltaster fructicola* were newly discovered SBFS fungi from the Midwest U.S. (5). *Peltaster fructicola* was found in WI, MO, and IL and occurs widely in North Carolina.

Preparation of inoculum. Mycelial cultures from -80°C storage were transferred to 1.5% malt extract agar (MEA), carnation leaf agar (CLA), or potato dextrose agar (PDA) and incubated at 25°C in the dark until conidia production for each isolate was abundant. These media were required to obtain adequate conidial production for silica gel storage. Cultures were then transferred to 1.5 ml of 10% sterile skim milk suspension and poured into two tubes containing 4.5 g of silica gel. Isolates in silica gel were frozen until used. *Dissoconium* sp. DS1 isolates were not stored in silica gel, however, since this fungus did not produce adequate amounts of conidia for silica gel storage. Instead, *Dissoconium* sp. DS1 mycelia were directly transferred from 15% glycerol at -80°C onto MEA.

Ten silica gel crystals of each SBFS isolate were transferred onto each of four MEA plates (100 x 15 mm) and incubated at 25°C in the dark for 4 weeks. Cultures were scraped using a flame-sterilized rubber policeman; mycelial fragments and conidia were suspended in tubes containing 5 ml of sterile deonized water and vortexed for 15 seconds. Afterward, 1 ml

of suspension was transferred with a pipette onto each of four MEA plates and spread with a sterile bent glass rod to obtain a uniform distribution of mycelial growth. Plates were incubated at 25°C in the dark for 3 weeks.

Transfer of inoculum onto 12-well plates. Wells (22 mm diameter) in 12-well plates (Corning Incorporated, Costar®, Corning, NY), were each filled with 2 ml of 2% water agar (WA) the day before use, and the plates were wrapped in cellophane to prevent drying. Each well of each plate was assigned a randomly selected number from 1 to 12.

Six-mm-diameter plugs containing similar amounts of fungal mycelium were obtained from 3-wk-old cultures on MEA using a #2 cork borer. Plugs were transferred mycelium-side down to the center of each well. Each plate was then wrapped with parafilm and incubated at 10, 15, 20, 25, 30, or 35°C for 8 weeks in the dark. Incubator temperatures were monitored with sensors (Watchdog Model 450, Spectrum Technologies Inc., Plainfield, IL). The experiment was completed once at the University of Wisconsin, Madison, WI (WI-1 trial) and twice at Iowa State University, Ames, IA (IA-1 and IA-2 trials).

Mycelial growth in response to temperature. Every 7 to 11 days, two measurements of colony diameter, at a 90-degree angle to each other, were made using a ruler under a dissecting microscope. The experiment was continued for 8 weeks or until the fastest-growing colonies reached 18 mm in diameter, whichever occurred first.

Mean diameter growth for each isolate was calculated by averaging colony diameter measurements and subtracting the plug diameter. Area under mycelial growth curve (AUMGC) was then calculated using colony diameter and time (days) after inoculation between two colony diameter measurements (8, 21). AUMGC estimates were calculated by $\sum^{n-1}_{i=1}[(y_{i+1}+y_i)/2][t_{i+1}-t_i]$, where: n= total number of observations, y_i = first colony diameter

measurement expressed in mm at the $_i$th observation, y_i+1 = second colony diameter measurement expressed in mm at the $_i$th observation, and $t_{i+1} - t_i$ = time (days after inoculation) at the $_i$th observation.

Experimental design and statistical analysis. The experimental design was a randomized complete block with six replications per temperature for the IA-1 and WI-1 trials and four replications for the IA-2 trial.

The data were analyzed by analysis of variance (ANOVA) using PROC MIXED on SAS (version 9.1, SAS Institute, Cary, NC). The response variable was AUMGC and mycelial growth at 5 weeks; fixed effects were trial, temperature, species, isolates nested within species, and the resultant interactions. The random effects were the replications nested to species, trial*species, trial*species*temperature, and isolate*replication nested to species, trial*species, and species*temperature (Table 2). Once it was determined that each trial was to be analyzed separately, the variance for species and isolate within species was estimated for each trial to determine whether to analyze species or individual isolates within species. For each isolate, temperature means were compared using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

Results

There were significant interactions ($P<0.0001$) among trials and among temperature, species, and isolate within species (Table 2). Therefore, data from each trial were analyzed and presented separately. Variability observed among species was similar to variability observed between isolates of each species for all three trials (Table 3). Therefore, we evaluated each isolate and did not average isolates within each species. Standard errors were

similar in magnitude for the different temperatures. Generally, mycelial growth in trials IA-1 and IA-2 was similar, whereas growth was somewhat greater in trial WI-1 (Figs. 1-6). Optimal temperature for mycelial growth was typically 20 to 25°C, with slower growth at 10, 15, 30°C and little growth at 35°C. These trends were consistent across most species and all three trials (Fig. 1).

Dissoconium sp. *DS1*. For isolate PEB4a, there was no significant difference in mycelial growth between 10 and 25°C in two of the three trials (WI-1 and IA-2) (Fig. 1). In the IA-1 trial, the maximum mycelial growth occurred at 20 and 25°C. For isolate MSTB3a, maximum mycelial growth occurred at 20°C in the IA-1 trial, 10-25°C in the IA-2 trial, and 15-25°C in the WI-1 trial. Neither isolate of *Dissoconium* sp. grew appreciably at 30 or 35°C (Fig. 1).

Colletogloeum sp. FG2. For isolate UIF1d, maximum mycelial growth occurred at 20 and 25°C in the IA-1 and WI-1 trials and 20°C in the IA-2 trial (Fig. 2). Isolate UMF2a had optimal mycelial growth at 20 and 25°C for two trials (IA-1 and WI-1) and 25°C for the IA-2 trial. Neither isolate of *Colletogloeum* sp. FG2 had appreciable growth at 35°C (Fig. 2).

Peltaster fructicola P1. For isolate GTE1a, maximum mycelial growth occurred at 20 and 25°C for both IA trials and 10 to 25°C for the WI-1 trial. Isolate Pf002 grew fastest at 20 and 25°C in all trials. There was significantly more growth at 15 than at 10, 30 or 35°C. For both isolates, growth at 30 and 35°C was considerably below the maximum.

Peltaster sp. P2. For isolate CUE2b, optimal temperature was 25°C for all three trials. For isolate GTE5a in the WI-1 trial, however, there was no significant difference in growth between 10 and 30°C.

Peltaster sp. CS1. For isolate PEE1, the optimal temperature for mycelial growth was 20°C for the IA-1 and IA-2 trials and 20 - 25°C for the WI-1 trial (Fig. 5). For isolate UIE17b, there was very little mycelial growth (<2 mm) at any temperature in the two IA trials. In the IA-1 and WI-1 trial there was no significant difference in mycelial growth between 10 and 30°C. For the IA-2 trial, the optimal temperatures for mycelial growth were 20 and 25°C. For both isolates, there was little or no growth at 35°C.

Pseudocercosporella sp. RH1. For isolates MWD2a and AHD1a, maximum mycelial growth occurred at 25°C in one trial and 20 to 25°C in two trials (Fig. 6). Growth was sharply reduced at 30°C and almost absent at 35°C.

Discussion

This is the first study characterizing the environmental biology of newly discovered SBFS species described by Batzer et al. (5). For the six species evaluated, optimal temperatures for mycelial growth was generally 20 to 25°C. Mycelial growth was generally slower at 10 to 15°C and sharply reduced at 35°C. Other studies found an optimal temperature range between 15 and 24°C for the previously named SBFS species *Gloeodes pomigena*, *Zygophiala jamaicensis, Peltaster fructicola,* and *Leptodontidium elatius* (2, 3, 15, 18, 21, 27).

Baines and Gardner (1932) established that the maximum mycelial growth for one species, *Gloeodes pomigena*, occurred between 18 and 27°C, with optimum growth at 20°C and no growth at 0 or above 30°C. Similarly, Groves (1933) reported no growth of *G. pomigena* above 30°C. Hickey (1960) reported that the optimum temperature range of *G. pomigena* was 20 to 29°C. Baines (1940) reported an optimum temperature range of 20 to

24°C for *Zygophiala jamaicensis*, with little growth at 27°C. In similar study, the optimum mycelial growth for *Z. jamaicensis* was reported at 16 to 24°C, with less growth at 12 and 28°C, and no growth at 8 and 32°C (27).

Johnson and Sutton (2000) reported that mycelial growth of *Leptodontidium elatius* was more tolerant to high temperatures than that of *Peltaster fructicola*. The authors reported little mycelial growth of *P. fructicola* at 32°C or higher, whereas the more tolerant *L. elatius* continued to grow at these high temperatures. In addition, an optimum temperature for mycelial growth for *P. fructicola* and *L. elatius* was established at 12 to 24°C and 16 to 28°C, respectively.

The temperature response of *G. pomigena*, *Z. jamaicensis, P. fructicola* and *L. elatius* from previous studies was consistent with our results. However, establishment of a consensus optimum temperature for mycelial growth of a particular species is confounded by different growth media, temperature ranges, and culture techniques among various studies. Thus, direct comparison among studies may reveal differences that are attributable to the specific conditions of each experiment.

Our findings are evidence that mycelial growth of most SBFS species tested responded similarly to temperature. However, considerable variation between isolates within species was observed. In contrast, sensitivity of mycelial growth to thiophanate-methyl and ziram fungicide did not differ between isolates of the same SBFS species described by Batzer et al. (33). Response of mycelial growth and sporulation to agar media varied among these putative species (34).

In recent years, integrated management of SBFS has been proposed based on warning systems to reduce frequency of fungicide sprays in apple orchards (7, 16, 17, 31). Validation

trials of warning systems in the North Central U.S. estimated a reduction of 2.5 fungicide applications per season compared to a conventional calendar-based spray schedule (1, 12, 13). Thus, prediction of SBFS growth based on warning systems requires adequate knowledge of the pathogen ecology. Our study estimated the optimum temperature for mycelial growth of important SBFS species in the Midwest, as a precursor to refinement of existing SBFS warning systems for use in the Midwest (7, 16, 17, 31).

Based on this study, as well as previous reports, we suggest that temperature may not be a significant factor in predicting SBFS mycelial growth, despite the diversity of species involved in the SBFS complex, because temperature optima were generally broad, and usually centered at 20-25 °C. Further studies of the ecology of SBFS should assess the interaction of relative humidity, leaf wetness duration and temperature on mycelial growth. Ultimately, a reliable warning system could assist apple growers in implementing alternative management strategies to improve the market value of apples and reduce the environmental impact of the disease.

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Tables

Table 1. Isolates of SBFS fungi complex used in the temperature experiment.

Species	Mycelial type [a]	Isolate designation	Origin (city or county, state)
Dissoconium sp. DS1	Discrete Speck	PEB4a	Pella, IA
Dissoconium sp. DS1	Discrete Speck	MSTB3a	New Muenster, WI
Colletogloeum sp. FG2	Fuliginous	UIF1d	Urbana, IL
Colletogloeum sp. FG2	Fuliginous	UMF2a	New Franklin, MO
Peltaster fructicola P1	Punctate	GTE1a	Chester, IL
Peltaster fructicola P1	Punctate	Pf002	Moore County, NC
Peltaster sp. P2	Punctate	CUE2b	Rockford, IL
Peltaster sp. P2	Punctate	GTE5a	Chester, IL
Peltaster sp. CS1	Compact speck	PEE1	Pella, IA
Peltaster sp. CS1	Compact speck	UIE17b	Simpson, IL
Pseudocercosporella sp. RH1	Ridged honeycomb	MWD2a	Indianola, IA
Pseudocercosporella sp. RH1	Ridged honeycomb	AHD1a	Mooresville, MO

[a] SBFS complex have been categorized into various mycelial types that vary morphologically among species.

Table 2. Analysis of variance of mycelial growth of 12 SBFS isolates at different temperatures (all three trials combined).

Source of variation	df	Pr > F
Trial	2	<0.0001
Species	5	<0.0001
Temperature	5	<0.0001
Trial*species	10	<0.0001
Trial*temperature	10	<0.0001
Species*temperature	25	<0.0001
Trial*species*temperature	50	<0.0001
Isolates (species)	6	0.0320
Isolates (trial*species)	12	<0.0001
Isolates (species*temperature)	30	<0.0001
Isolates (species*temperature*trial)	60	<0.0001
Replication (species)	30	0.3250
Replication (trial*species)	48	0.9630
Replication (species*temperature)	150	0.8062
Replication (trial*species*temperature)	240	0.4410
Isolates*replication (species)	30	0.4560
Isolates*replication (trial*species)	48	<0.0001
Isolates*replication (species*temperature)	150	0.4701
Residual	240	

Table 3. F values for 12 SBFS isolates at different temperatures (for each trial).

		F value [a]		
Source of variation	**df**	**IA-1**	**WI-1**	**IA-2**
Temperature	5	594.9	339.1	176.0
Species	5	223.2	82.6	18.7
Isolates (species)	6	114.8	56.0	14.3
Temperature*species	25	36.9	13.3	5.4
Isolates (species*temperature)	30	15.5	7.1	5.5

[a] All were significantly different at $P<0.0001$

Figures

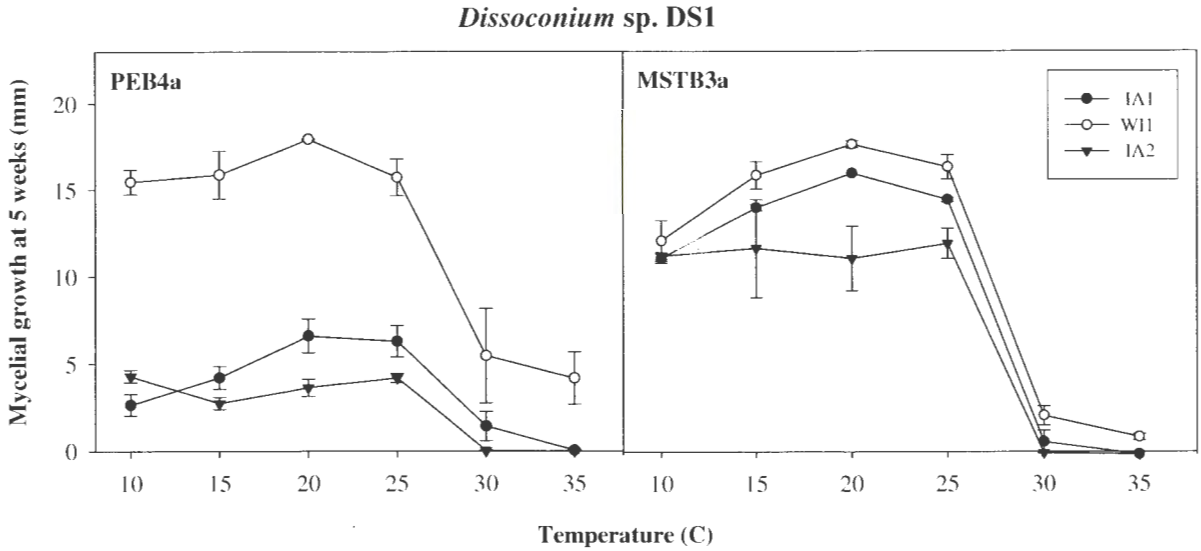

Figure 1. Mycelial growth of two *Dissoconium* sp. DS1 isolates at six different temperatures. Results of three separate trials (IA-1, WI-1, and IA-2) are shown.

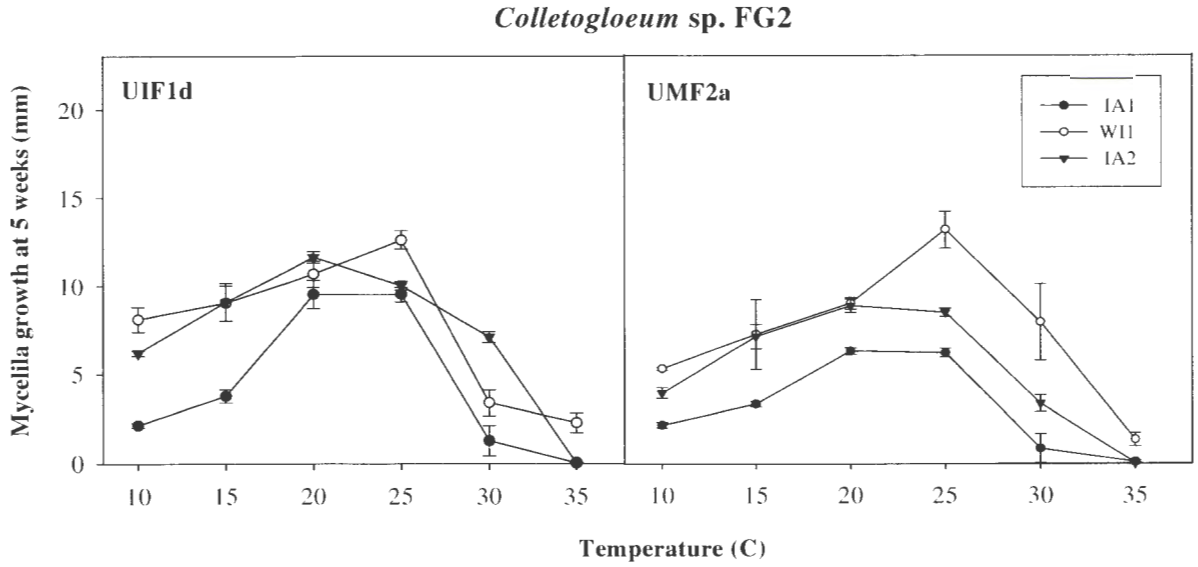

Figure 2. Mycelial growth of two *Colletogloeum* sp. FG2 isolates at six different temperatures. Results of three separate trials (IA-1, WI-1, and IA-2) are shown.

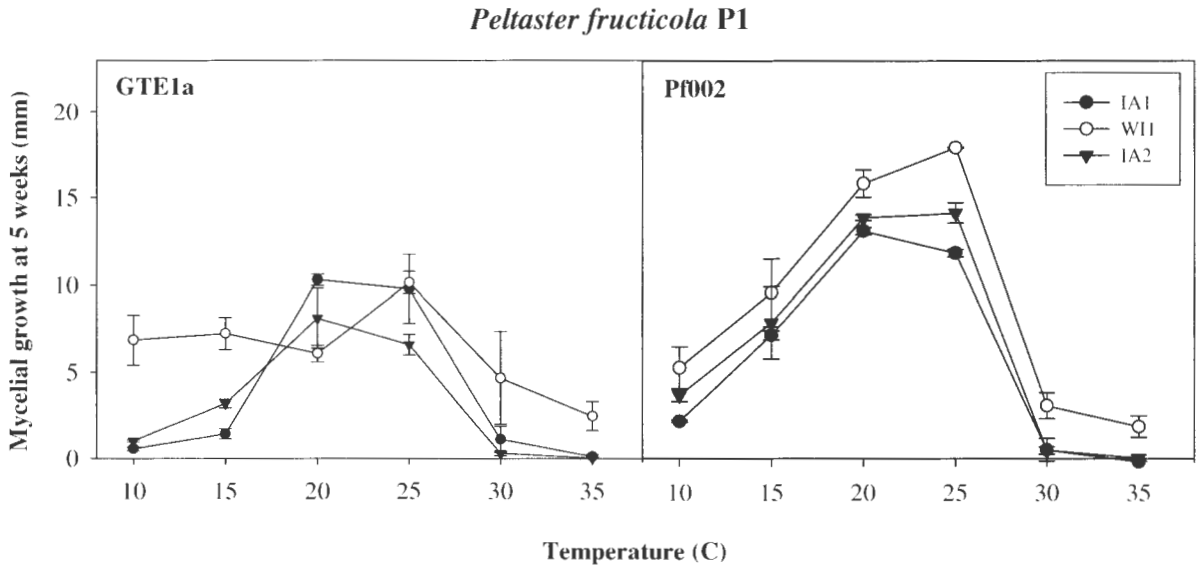

Figure 3. Mycelial growth of two *Peltaster fructicola* P1 isolates at six different temperatures. Results of three separate trials (IA-1, WI-1, and IA-2) are shown.

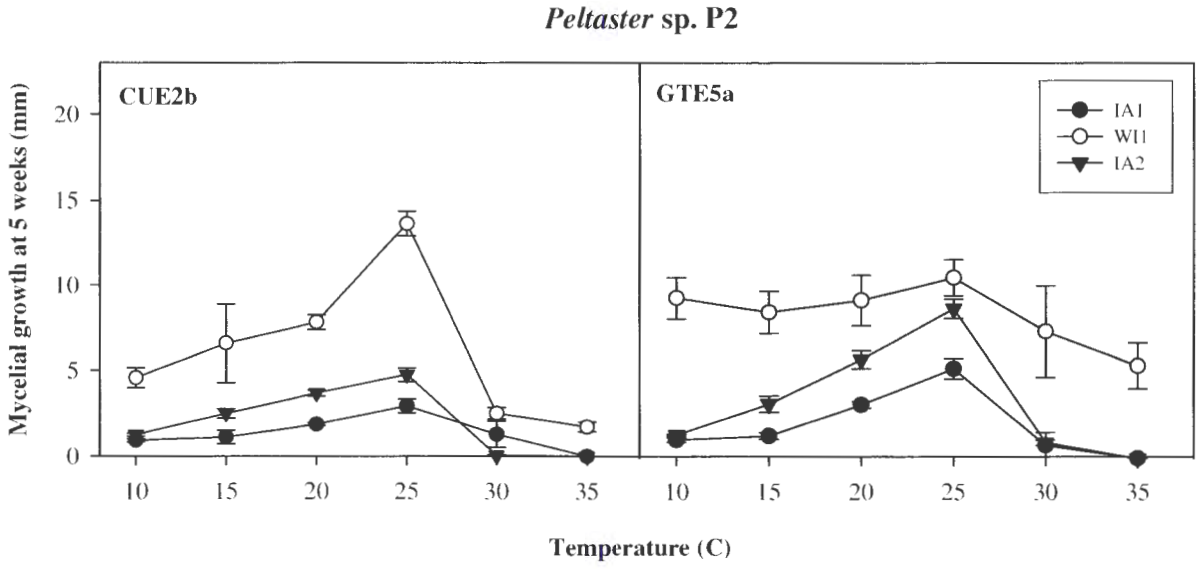

Figure 4. Mycelial growth of two *Peltaster* sp. P2 isolates at six different temperatures. Results of three separate trials (IA-1, WI-1, and IA-2) are shown.

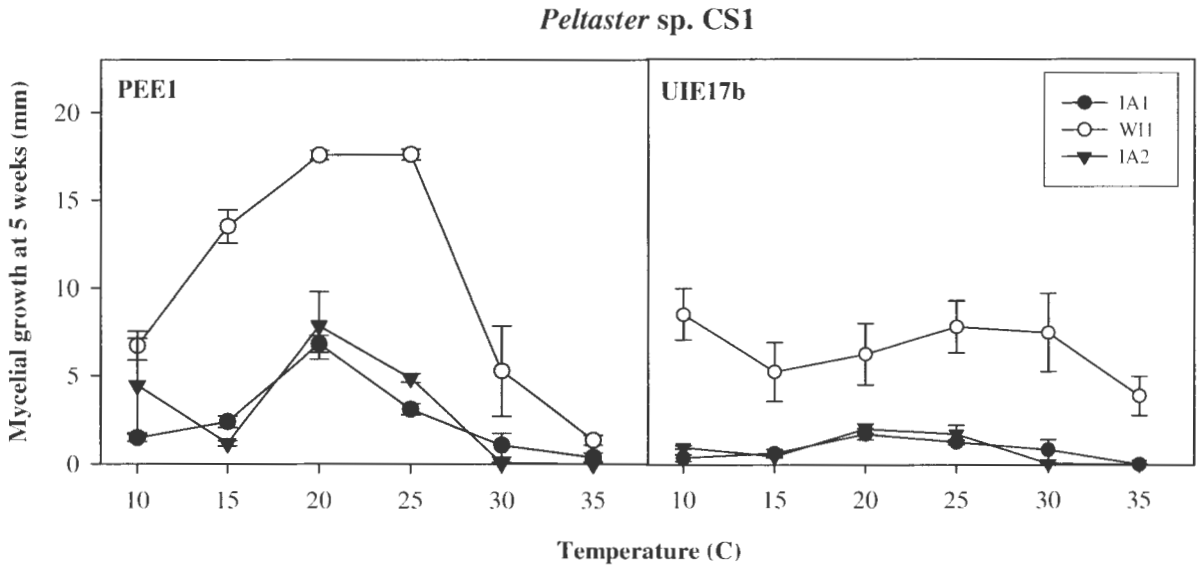

Figure 5. Mycelial growth of two *Peltaster* sp. CS1 isolates at six different temperatures. Results of three separate trials (IA-1, WI-1, and IA-2) are shown.

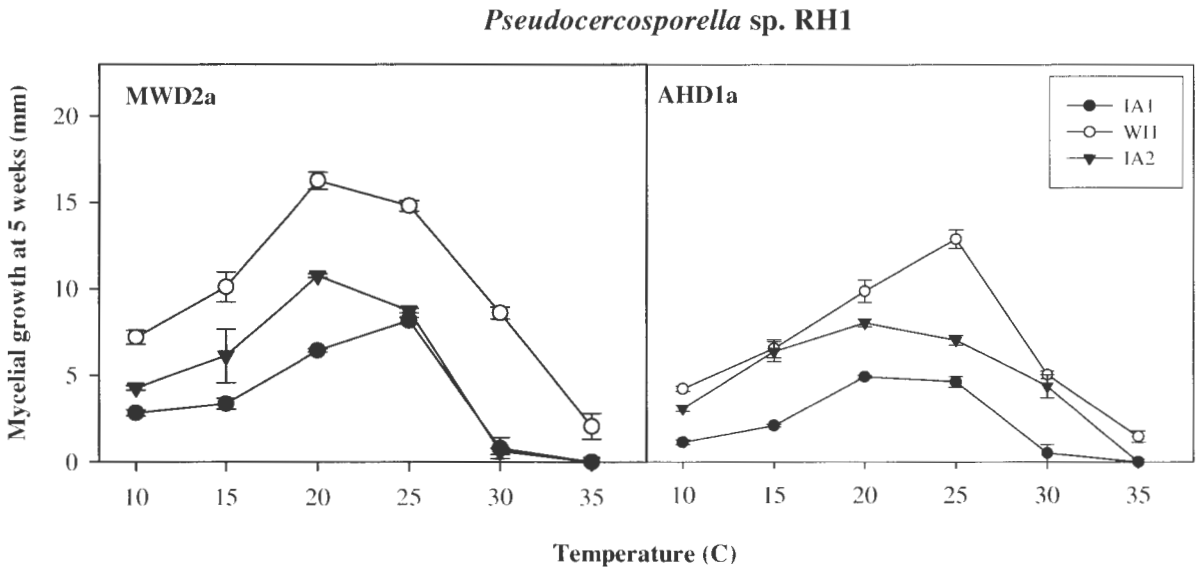

Figure 6. Mycelial growth of two *Pseudocercosporella* sp. RH1 isolates at six different temperatures. Results of three separate trials (IA-1, WI-1, and IA-2) are shown.

CHAPTER 3. POST-HARVEST REMOVAL OF SOOTY BLOTCH AND FLYSPECK ON APPLES BY BRUSHING COMBINED WITH DIP TREATMENTS

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Abstract

Sooty blotch and flyspeck (SBFS) fungi colonize the cuticle and reduce the quality and value of apples. One feasible alternative to reduce SBFS injury to market-acceptable levels is removal of the blotches by post-harvest washing and brushing. This study compared post-harvest dip treatments (NaOCl at 200 and 500 µg/ml, ClO_2 at 1 and 5 µg/ml, and fruit soap), followed by brushing on a grading line, for removal of SBFS. After a 7-min dip treatment, apples were brushed for 15, 30, 60, or 90 s on a grading line. Percent disease was determined before and after treatment of 'Honey Gold' apples from Iowa and Wisconsin in 2002 and 'Golden Delicious' apples from Kentucky and North Carolina in 2003. Removal of SBFS in all treatments was variable, but generally exceeded the no-dip control. Increasing the brushing time significantly increased removal of SBFS signs. After further optimization, post-harvest treatments therefore may provide growers with viable alternatives for improving appearance of SBFS- blemished apples to meet fresh-market standards.

Introduction

The sooty blotch and flyspeck (SBFS) disease complex is a group of saprophytic fungi that colonizes the cuticle of apples (*Malus* x *domestica* Borkh) and pears (*Pyrus communis* L.). Sooty blotch is caused by several species, including *Leptodontidium elatius* (G. Mangenot) de Hoog, *Peltaster fructicola* Johnson, Sutton & Hodges, and *Geastrumia polystigmatis* Batista & M. L. Farr., whereas flyspeck is caused by *Zygophiala jamaicensis*

Mason. Recently, Batzer et al. (3) described 30 putative species of SBFS from nine Midwest U.S. apple orchards and also noted the occurrence of *Peltaster fructicola* and *Zygophiala jamaicensis* in these orchards.

The SBFS complex damages the appearance of fruits and reduces the quality and value of apples. All cultivars of apples are considered susceptible to SBFS (10, 13, 23). Sooty blotch signs appear as dark olive green to brown blemishes (7, 15), whereas flyspeck appears as groups of tiny, round to irregular-shaped, shiny black specks on the epicuticular wax layer of apples (6, 9, 16, 18, 19).

SBFS can reduce the fresh market value of apples by more than 90% (25). For example, a bushel of certain varieties of apples may sell for as much as $32 on the fresh market, whereas a bushel of apples affected by SBFS, which are suitable for cider production, may only sell for as little as $2 (2). In addition to the cosmetic effects caused by SBFS, the disease can produce indirect losses due to the cost of frequent applications of protectant fungicides (21). In order to suppress SBFS, producers typically spray fungicides every 10 to 14 days, beginning at the first-cover stage and concluding shortly before apple harvest (4, 11, 12).

In warm, humid regions of the U.S., weekly applications of fungicides are sometimes inadequate to control SBFS, resulting in a 5 to 10% annual reduction in marketable apples (17). In the Southeast U.S., losses of almost 100% due to SBFS were observed in orchards where protectant fungicides were not applied (24).

As a result of significant quality losses in pome fruits, researchers have proposed an integrated approach for management of SBFS (14). SBFS has historically been managed with protectant fungicides; however, apple growers have experienced control failures due to

inadequate fungicide coverage, deficient pruning, and disease-favorable environments (8, 15).

Post-harvest washing and brushing treatments have been suggested as viable alternatives for removal of SBFS signs. Post-harvest SBFS removal tactics could compensate for inadequate fungicide control in the field and potentially reduce reliance of apple growers on fungicide sprays.

Chlorine dioxide (ClO_2), sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), peroxyacetic acid, and fruit soap are sanitizers that can increase fruit quality and value through removal of bacteria and fungi (2, 5, 12, 22, 25). Sodium hypochlorite has been cited as an effective dip treatment to reduce SBFS severity (2, 7, 12, 25). Colby (7) recommended 3- to 6- minute dips of apples in Javelle water (sodium hypochlorite), followed by rinsing in tap water, for removal of SBFS signs.

Baker (1) first reported the use of chlorine to reduce apple decay. Doses of chlorine at 50 µg/ml and chlorine dioxide at 10 µg/ml significantly reduced conidial germination of *Botrytis cinerea*, *Mucor piriformis*, and *Penicilium expansus* in d' Anjou pears (22). Hendrix (12) reported a reduction of sooty blotch incidence from 100 to 0% and flyspeck from 100 to 27% when apples were dipped in 500 µg/ml of NaOCl for 5 to 7 minutes followed by brushing. Therefore, chlorine dips could serve a dual purpose: removal of SBFS signs and suppression of post-harvest decay fungi. However, post-harvest dip treatments are not routinely used by many small-scale apple growers in the Midwest U.S. The effectiveness of sanitizer concentration and dip time (7 or 15 min) of 200, 400, 500, 600, or 800 µg ml^{-1} NaOCl (Agclor 310 plus Decco 312 Buffer), a mixture of hydrogen peroxide and peroxyacetic acid (Tsunami 100) at 60 µg ml^{-1}/80 µg ml^{-1}, 120 µg ml^{-1}/160 µg ml^{-1}, or 360 µg

ml^{-1}/480 μg ml^{-1}, respectively, and fruit soap (Kleen 440) were evaluated as removal treatments on 'Golden Delicious' and 'Jonathan' apples (2). A 7-min dip of 800 μg ml^{-1} NaOCl increased market value of Jonathan apples by 14% and 'Golden Delicious' by 31%, and a 200 μg ml^{-1} NaOCl dip resulted in 28 and 45% increases in market value for Jonathan and McIntosh, respectively (2).

The purpose of this study was to assess efficacy of five post-harvest dip solutions (NaOCl at 200 and 500 μg ml^{-1}, ClO2 at 1 and 5 μg ml^{-1}, and fruit soap) followed by four brushing times (15, 30, 60 or 90 s) on removal of SBFS signs from apples. The information generated may help growers by providing alternatives for post-harvest removal of SBFS and thus enhancing the fresh-market value of apples.

Materials and methods

From September 25 to 29, 2002, 2,000 'Honey Gold' apples with SBFS signs were harvested from Wilson's Orchard in Iowa City, IA, and from Bohl's Orchard in Madison, WI. From September 20 to 27, 2003, 4,000 'Golden Delicious' apples were harvested from the Browning Orchard in Wallingford, KY, and from North Carolina State University's Mountain Horticultural Crops Research Station in Fletcher, NC.

SBFS severity varied among locations. 'Honey Gold' apples from IA and WI were lightly blemished with SBFS, whereas 'Golden Delicious' apples harvested at the NC and KY orchards exhibited severe SBFS signs, in addition to russeting and black pox (pathogen: *Helminthosporium papulosum*) symptoms. The difference in severity was attributable to difference in prevailing weather conditions at the orchards during 2002 and 2003. The low severity of SBFS in IA and WI apples was attributed to relatively dry conditions in 2002,

whereas favorable field weather conditions for SBFS development was experienced in the Southeast during 2003.

Apples with uniform size and ripeness, and having at least 3% of the fruit surface covered by SBFS signs, were selected. Bruised or defective fruit were rejected. Harvested apples were placed in wooden crates and transported to the Iowa State University Horticulture Research Farm. Fruit were stored in coolers at 4°C for 3 weeks, until treatments were applied.

Five dip treatments were tested: 200 and 500 μg ml^{-1} sodium hypochlorite (NaOCl) (Decco Agclor 310 / 312 Buffer concentrate) [Cerexagri, Inc., Monrovia, CA], 1 and 5 μg ml^{-1} chlorine dioxide (ClO_2) [Intellectual Capital Associates (ICA) TriNova, LLC, Forest Park, Georgia] and fruit soap solution (NaOH) (Decco Fruit & Vegetable Kleen 440) [Cerexagri, Inc.]. Immediately following the dip treatment, apples were brushed by nylon brushes for 15, 30, 60, or 90 s on a commercial grading line. A control treatment received no dipping, but underwent brushing.

Several procedures were adopted to enhance accuracy of pre- and post-treatment rating of SBFS severity. An indelible marker was used to draw a line from the stem end to the calyx, dividing the apple vertically into halves. In each year, ten apples (five from each orchard) were arbitrarily selected, placed in plastic bags labeled with the brushing time, dip treatment, and replication number, and stored in plastic crates at 4°C. Before and after dip treatments were applied, percent area covered by SBFS signs on the front and back halves of each apple was estimated with reference to a standard area diagram (2).

Dip solutions were prepared 30 min prior to initiation of each trial. Solutions were prepared in 31 liters of tap water in a polypropylene tank. One day before ClO_2 was applied,

the treatment solution was titrated according to a method suggested by ICA TriNova (Joel Tenney, *personal communication*).

Subsamples of apples were placed in dip treatments and rotated frequently to ensure adequate coverage. After 7 min, apples were removed from the dump tank with a dip net, placed on a canvas conveyor belt leading to a commercial grading line, and subjected to 15-, 30-, 60-, or 90-s exposure periods on nylon brushes. Apples were then rinsed with tap water under spray nozzles for 20 seconds and pushed manually over wooden rollers. Finally, apples were replaced into plastic bags and stored at 4°C until percent disease area was re-estimated.

The experimental design was a complete randomized design with six replications in each year. Two replications per day were conducted for three consecutive days at the Iowa State University Horticulture Research Farm. The percentage of SBFS removal was calculated by averaging estimates for the front and back halves of each apple before and after dip treatments.

Data were analyzed by analysis of variance (ANOVA) in SAS (version 9.1, SAS Institute, Cary, NC). The response variable was the percentage of SBFS removal; fixed effects were orchard locations, dip treatments, brushing times, and resultant interactions (Table 1). Means of SBFS percentage removal were compared with Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ to determine differences among dip treatments and brushing times.

Results

Since different apple cultivars were used in the 2002 and 2003 trials and no significant differences between orchards within year were observed, data are presented separately for each year (Table 1).

Effects of brushing time on removal of SBFS signs. Efficacy of SBFS removal depended on duration of brushing on the grading line (P = <0.0001) (Fig. 1A, Table 1). Brushing times of 15 and 30 s did not differ significantly in reducing percent SBFS on apples in either year. However, these two treatments differed significantly from the 60- and 90- s brushing times. In general, the most efficient brushing time was 90 s, removing >85% of SBFS signs in 2002 and >68% in 2003.

Effects of five dip treatments on removal of SBFS signs. Generally, the dip treatments were more effective at removing SBFS signs from 'Honey Gold' apples in 2002 than for 'Golden Delicious' in 2003 (Fig. 1B). Removal of SBFS signs varied among dip treatments across locations, but generally exceeded removal by the no-dip control for all four orchards.

While fruit soap tended to be the most effective dip treatment, removing >85% of SBFS signs, no significant difference between this treatment and the no-dip control occurred in 2003. In 2002, 5 μg ml^{-1} ClO_2 removed 83% SBFS signs from 'Honey Gold' apples; however, the same treatment removed 65% of SBFS signs from 'Golden Delicious' apples in 2003. NaOCl at 500 μg ml^{-1} was the most efficient treatment in 2003, removing 78% of SBFS signs.

Analysis of variance identified no significant interactions for orchard*dip treatment, orchard*brushing, or dip treatments*brushing interactions in the 2002 and 2003 trials (Table 1). In contrast, a significant difference was observed for the orchard*dip treatment*brushing

interaction in 2003 evaluations (Table 1).

Discussion

Our study is the first to document the impact of brushing time on post-harvest removal of SBFS signs. Our results suggest that growers could improve SBFS removal efficacy by either detaining apples on a brushing table or installing additional brushing tables. However, variability in efficacy of post-harvest SBFS removal suggests that only relatively lightly blemished apples are likely to receive economically significant benefits from the treatments tested in this study.

Fruit soap was found to be the most effective dip treatment, removing >85% of SBFS signs on 'Honey Gold' apples from Wisconsin and Iowa orchards in 2002. However, the soap treatment was less effective in 2003. Lower efficacy in 2003 was attributable to the high level of SBFS severity of these apples. Fruit soaps are surfactants that remove superficial SBFS signs, and thus are apparently more effective at low disease severity. Batzer et al. (2) reported that soap removed 90% of SBFS signs from lightly infested 'McIntosh' apples and 62% from lightly infested 'Golden Delicious' apples.

In contrast to fruit soap, 500 μg ml^{-1} NaOCl was the most effective dip treatment in 2003, removing >78% SBFS signs from heavily infested 'Golden Delicious' apples. Sodium hypochlorite at 200 μg ml^{-1} was also effective, resulting in >78% SBFS removal in the 2002 trial and >65% in the 2003 trial. Similarly, Batzer et al. (2) reported that concentrations of 500 and 800 μg ml^{-1} NaOCl were more effective dip treatments for removal of SBFS from apples than 200 μg ml^{-1} NaOCl. In another study, Hendrix (12) reported that the incidence of sooty blotch was reduced from 100 to 0% and flyspeck from 100 to 27% when apples were

dipped in 500 μg ml^{-1} of NaOCl combined for 5 to 7 minutes, followed by brushing. In the same experiment, sooty blotch was reduced from 100 to 60% following treatment with 200 μg ml^{-1} of NaOCl.

Chlorine dioxide at 1 and 5 μg ml^{-1} was effective in removing SBFS signs in 2002. In 2003, however, these concentrations were less efficient for SBFS removal from 'Golden Delicious' apples. These results suggest that the ClO_2 concentrations used were most effective for apples with low severity of SBFS. Compared to NaOCl, treatments with ClO_2 have a potential advantage since the chemical is less sensitive to pH and organic matter, and poses less health risk to workers (20).

Differences in effectiveness of dip treatments and brushing between 2002 to 2003 trials could be attributed to differences in apple cultivars and in environmental conditions during the growing seasons. In 2002, physical appearance of most apples was acceptable after the dip and bushing treatments, suggesting potential to affect in fresh-market value. Dip treatments and brushing therefore could eventually become acceptable alternatives to remove SBFS signs, particularly in apples with relatively mild infestations of SBFS fungi. Additional research is needed to optimize efficacy of dip treatments and post-harvest brushing on removal SBFS from apples.

Acknowledgments

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Tables

Table 1. Analysis of variance of post-harvest removal of SBFS by brushing combined with dip treatments on 'Honey Gold' apples harvested from Iowa and Wisconsin orchards in 2002 and 'Golden Delicious' apples harvested from Kentucky and North Carolina orchards in 2003.

Source of variation	df	2002 Pr >F	2003 Pr >F
Orchards	1	0.7030	0.2709
Dip treatments	5	<0.0001	<0.0001
Brushing times	3	<0.0001	<0.0001
Dip treatments*orchards	5	0.4896	0.7148
Brushing*orchards	3	0.7144	0.2713
Dip treatments*brushing	15	0.5849	0.2189
Dip treatments*brushing*orchards	15	0.8451	0.0275
Mean Square Error	1052	297.44	538.89

Figures

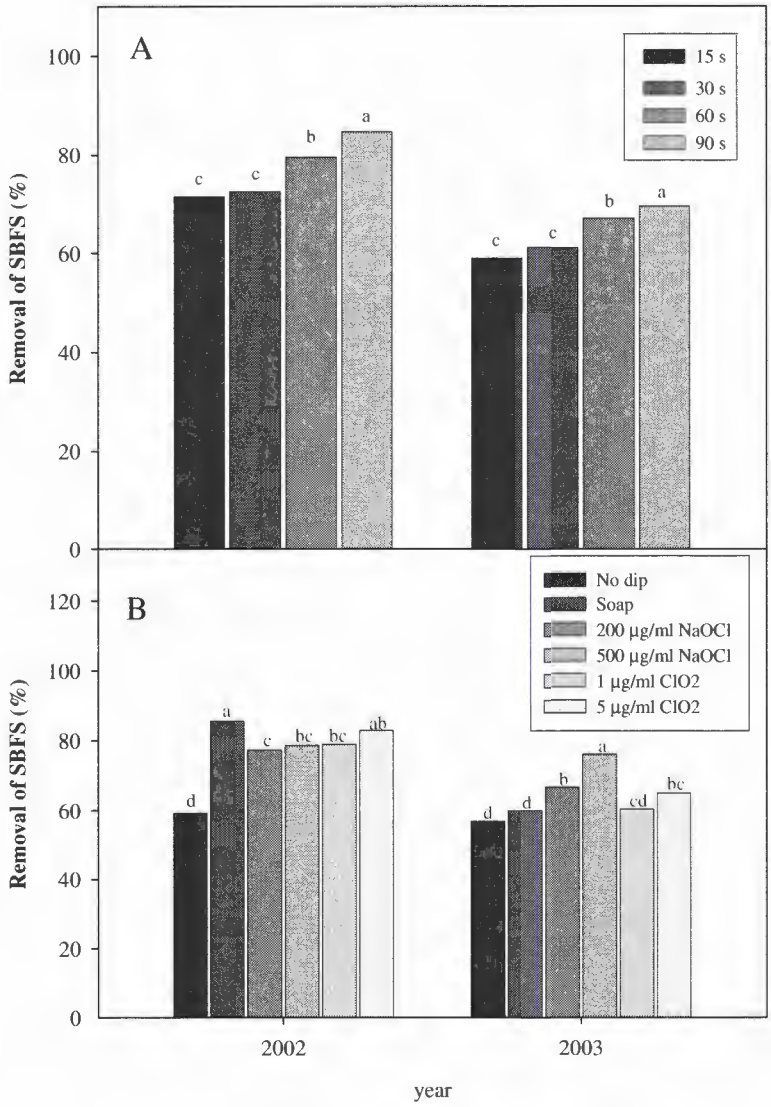

Figure 1. Removal of SBFS using four brushing times (A) and five dip treatments (B) on 'Honey Gold' apples from orchards in Iowa and Wisconsin in 2002 and 'Golden Delicious' apples from orchards in Kentucky and North Carolina in 2003. Each bar represents the mean of six replications, two orchards, and four brushing times (n = 48) (A) and six replications, two orchards, five dip treatments, and no-dip control (n = 72) (B). Bars with the same letters within year are not significant different at $P > 0.05$ according to Fisher's LSD test.

CHAPTER 4. GENERAL CONCLUSIONS

The sooty blotch and flyspeck (SBFS) is a serious disease complex of apples (*Malus* x *domestica* Borkh) during summer months in the Midwest U.S. Conventional management practices often requires 3 to 8 fungicides sprays on a calendar-based schedule, initiating with the first-cover stage and ending shortly before harvest (25). In addition to the cost of frequent applications of protectant fungicides, SBFS reduces the fresh market values of apples due to the undesirable appearance of infested fruit; the resultant low acceptance by the consumer typically renders fruit to cider production (7).

Until recently, only four SBFS species were known including *Leptodontidium elatius* (G. Mangenot) de Hoog, *Peltaster fructicola* Johnson, Sutton & Hodges and, *Geastrumia polystigmatis* Batista & M. L. Farr., and *Zygophiala jamaicensis* Mason. Recently, Batzer (2005) identified 30 new species of SBFS from the Midwest U.S. The research described in this thesis provided information about the optimal temperature for mycelial growth of two isolates of each of the five newly discovered SBFS species in the Midewst U.S. (*Dissoconium* sp. DS1, *Colletogloeum* sp. FG2, *Peltaster* sp. P2, *Peltaster* sp. CS1, and *Pseudocercosporella* sp. RH1) and one previously described species from North Carolina (*Peltaster fructicola* P1). Generally, the optimum temperature for mycelial growth occurred from 20 to 25°C for all six species, with slower growth 10 and 15°C and little to no growth at 30 or 35°C. Temperature requirements for growth increased our understanding of the SBFS complex ecology and potentially assisted in developing management practices to effectively manage the fungus complex in the field. Further studies of the ecology of SBFS should

assess the interaction of relative humidity and temperature on mycelial growth of the new species identified in the Midwest.

The second study assessed the feasibility of post-harvest washing and brushing as means to reduce SBFS signs on apples and increase the market acceptance. This study was the first to document the impact of brushing time on post-harvest removal of SBFS signs. The results of this study suggest that post-harvest dip treatments (NaOCl at 200 and 500 µg/ml, ClO_2 at 1 and 5 µg/ml, and fruit soap) followed by 60 or 90 s of effectively removed SBFS signs on commercial apples. However, variability in efficacy of post-harvest SBFS removal suggests that only the less blemished apples are likely to receive economically significant benefits from the treatments tested in this study. Post-harvest SBFS removal tactics could compensate for inadequate fungicide efficacy in the field and potentially reduce reliance of apple growers on fungicide sprays. In addition, post-harvest removal tactics may provide growers with alternatives for improving the appearance of SBFS–blemished apples thus potentially meet with fresh-market standards. More research is needed to optimize efficacy of dip treatments and post-harvest brushing for removal of SBFS signs on apples.

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